

What is claimed is:

1. A method for discriminating dysplastic cells over-expressing INK4a gene products from other cells expressing INK4a gene products at a detectable level in biological samples comprising determining in a cytological or histological testing procedure the co-expression of at least two marker molecules in at least one single cell, wherein at least one marker molecule is an expression product encoded by the INK4a gene and at least one further marker molecule is a cell proliferation marker, wherein the over-expression of at least one INK4a gene product and expression of at least one marker for active cell proliferation at a detectable level within said single cell is indicative of the dysplastic state of the cell and wherein the over-expression of at least one INK4a gene product and expression of at least one marker for senescence, terminal cell differentiation, apoptosis or cell cycle arrest at a detectable level within said single cell is indicative of the non-dysplastic state of the cell.
2. A method according to claim 1, wherein a set of two or more cell proliferation markers is detected.
3. A method according to claim 1 or 2, wherein at least one INK4a gene-product has a molecular weight between 13 and 19 kDa.
4. A method according to claims 1 to 3, wherein at least one INK4a gene-product is selected from a group comprising p16^{INK4a} and p14ARF.
5. A method according to any one of the preceding claims, wherein at least one cell proliferation marker is selected from a group comprising a proliferation marker necessary for the maintenance of cell proliferation, a proliferation marker engaged in DNA replication, a proliferation marker being or encoding a member of the processive replication fork, a senescence marker, a cell cycle arrest marker and an apoptosis marker.
6. The method according to claim 5, wherein the gene-product necessary for the maintenance of cell proliferation is a molecule selected from a group comprising Ki67 molecules, Ki-S5 molecules and Ki-S2 molecules.
7. The method according to claim 6, wherein the gene-product engaged in DNA replication is selected from a group comprising helicases or subunits thereof, cell division cycle (cdc) molecules, phosphatase molecules and kinase molecules.
8. The method according to claim 7, wherein the helicases or subunits thereof are selected from a group comprising MCM2, MCM3, MCM4, MCM5, MCM6, MCM7 and HELAD1.

9. The method according to claim 7, wherein the cdc molecules, kinases and phosphatases are selected from a group comprising CDC6, CDC7 protein kinase, Dbf4, CDC14 protein phosphatase, CDC45 and MCM10.
- 5 10. The method according to claim 5, wherein the molecules engaged in the processive replication fork is selected from a group comprising PCNA and POLD.
11. A method according to any one of the claims 1-10, wherein the gene-product is a polypeptide or a nucleic acid molecule.
12. A method according to any one of the preceding claims, wherein additionally at least one further marker molecule is detected for improvement of the assessment of diagnosis or prognosis.
- 10 13. A method according to claim 12, wherein the further marker molecule is at least one further proliferation marker molecule.
14. A method according to claim 12 or 13, wherein the further marker molecule is selected from a group comprising a senescence marker, an apoptosis marker, a cell cycle arrest marker, a marker for terminal differentiation of cells, a marker for viral infection, a marker for viral activity, a cell cycle regulatory protein, a gene-product necessary for the maintenance of cell proliferation, a gene-product engaged in DNA replication, a gene-product being a member of the processive replication fork.
- 15 15. A method according to any one of the preceding claims, wherein additionally a cytological staining procedure employing at least one dye selected from a group comprising DAPI, Quinacrin, Chromomycin, Azan, Acridin-orange, Hematoxylin, Eosin, Sudan-red, Toluidin-blue, and Thionin, or a staining method selected from a group comprising Pap-staining, Giemsa-staining, Hematoxylin-Eosin staining, van-Gieson-staining, Schiff-staining, staining via metal precipitates, Turnbulls-blue-staining and staining via metal cyanides is applied.
- 20 16. The method according to any one of the preceding claims, wherein the dysplastic cells are cells of a cancerous or precancerous lesion.
- 25 17. The method according to claim 16, wherein the dysplastic cells are cells of a dysplasia being associated with a papilloma virus.
18. The method according to claim 17, wherein the papilloma virus is high risk human papilloma virus selected from a group comprising HPV16, HPV18, HPV31, HPV 33, HPV35, HPV 39, HPV 45, HPV 51, HPV 52, HPV56, HPV 58, HPV 59, HPV 66 and HPV 68.
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19. The method according to any one of the claims 16 to 18, wherein the lesion is selected from a group comprising a lesion of the anogenital tract, a lesion of the respiratory tract and a lesion of the skin and its appendages.
- 5 20. The method according to claim 17 or 19, wherein the lesion is selected from a group comprising a lesion of the uterine cervix, a lesion of the vagina, a lesion of the vulva, a lesion of the penis, a lesion of the anus, a lesion of the rectum, a lesion of the bronchic tree, a lesion of the lung, a lesion of the peritoneal space, a lesion of the naso-pharyngeal space, a lesion of the oral cavity or a lesion of the skin.
- 10 21. A method according to any preceding claim, wherein the biological sample is a sample containing cells originating from the anogenital tract, from the respiratory tract or from the skin and its appendages .
22. A method according to claim 21, wherein the cells are cells originating from the uterine cervix, the vagina, the vulva, the penis, the anus, the rectum, the bronchic tree, the lung, the naso-pharyngeal space, the oral cavity or the skin.
- 15 23. A method according to any one of the preceding claims, wherein the biological sample is a cytological or histological preparation.
24. A method according to any one of the preceding claims, wherein the detection of the INK4a gene-products and/or the cell proliferation marker molecules is performed using at least one probe specifically recognising at least one of the respective molecules to be detected.
- 20 25. A method according to claim 24, wherein at least one probe is detectably labelled.
26. A method according to claim 25, wherein at least one label is selected from the group consisting of a radioisotope, a bioluminescent compound, a chemiluminescent compound, a fluorescent compound, a metal chelate, or an enzyme.
- 25 27. A method according to any one of the claims 24 to 26, wherein at least one probe is a protein and/or a nucleic acid.
28. A method according to claim 27, wherein at least one probe is an antibody directed against a INK4a encoded gene-product or a cell proliferation marker gene product.
29. The method according to claim 28, which comprises an immuno-cytochemical staining procedure.
- 30 30. The method according to claim 25 or 26, wherein at least one probe is a nucleic acid specifically hybridizing to an INK4a gene-product or a cell proliferation marker gene product.

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31. The method according to claim 30, which comprises an in situ hybridization reaction.

32. The method according to claim 30, which comprises a nucleic acid amplification reaction.

33. The method according to claim 32, wherein the nucleic acid amplification reaction is PCR, NASBA or LCR.

5 34. A method according to any of the preceding claims, wherein detection reactions using nucleic acid probes and polypeptide probes are carried out simultaneously.

35. A kit for performing the method according to any one of the preceding claims, which is a diagnostic kit or a research kit, comprising at least one or more probes for the detection of the presence or absence and/or the level of the over-expression of at least one INK4a gene-product and at least one cell proliferation marker gene product in biological samples.

10 36. A kit according to claim 35, wherein the INK4a gene products are selected from a group comprising p16^{INK4a} and p14ARF.

37. A kit according to claim 35 or 36, wherein the cell proliferation marker gene products are selected from a group comprising CDC6, MCM3, MCM3, MCM4, MCM5, MCM6, MCM7, CDC7 protein kinase, Dbf4, CDC14 protein phosphatase, CDC45 and MCM10, Ki67, Ki-S2, PCNA or POLD.

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38. The kit according to claims 35 to 37 furthermore comprising at least one of the following

a. a p16^{INK4a} sample for carrying out a positive control reaction

b. a p14ARF sample for carrying out a positive control reaction

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c. a Ki67 sample for carrying out a positive control reaction

d. a Ki-S2 sample for carrying out a positive control reaction

e. an MCM5 sample for carrying out a positive control reaction

f. an MCM2 sample for carrying out a positive control reaction

g. a PCNA sample for carrying out a positive control reaction

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h. reagents for detection of the presence or absence and/or the level of p16^{INK4a}

i. reagents for detection of the presence or absence and/or the level of p14ARF

j. reagents for detection of the presence or absence and/or the level of Ki67

k. reagents for detection of the presence or absence and/or the level of Ki-S2

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- l. reagents for detection of the presence or absence and/or the level of MCM5
- m. reagents for detection of the presence or absence and/or the level of MCM2
- n. reagents for detection of the presence or absence and/or the level of PCNA
- o. one or more samples of INK4a gene-products for carrying out positive control reactions
- 5 p. one or more samples of cell proliferation marker gene-products for carrying out positive control reactions
- q. one or more reagents for the detection of the presence or absence and/or the level of other INK4a gene products
- 10 r. and one or more reagents for the detection of the presence or absence and/or the level of other cell proliferation marker gene products.